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BIOENGINEERING GENERATION OF HEART RHYTHM: THE ROLE OF BIOPACEMAKER CELLS

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This review summarizes the anatomical and physiological characteristics of the sinoatrial node as a model for biopacemakers and analyzes current approaches to their development, including sinoatrial node cell transplantation, the use of pluripotent stem cells, gene therapy (HCN overexpression, IK1 suppression, TBX18 transduction, and others), as well as combined and reprogramming strategies Special attention is given to the authors' own experience and achievements in tissue engineering. Key obstacles to clinical translation are outlined, including safety concerns, stability of therapeutic effects, adequacy of experimental models, and control of biological pacemaker activity. Despite existing limitations, progress in gene engineering, stem cell technologies, and biomaterials offers promising opportunities for the development of fully physiological alternatives to electronic pacemakers.

Key words: bradyarrhythmia; pacemaker; artificial; biological pacemakers; genetic engineering; stem cells

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Bradyarrhythmias, including sick sinus syndrome and atrioventricular blocks, remain a leading cause of sudden cardiac death and impaired quality of life [1, 2]. According to the data of the Profile Commission on "Arrhythmology" of the Ministry of Health of Russia and the Bakulev National Medical Research Center for Cardiovascular Surgery, 53,386 permanent pacemaker (PPM) implantations were performed in the Russian Federation in 2023, corresponding to a rate of 364.9 procedures per 1 million population, reflecting a further increase in pacing volumes compared with 2021 [3]. Despite significant technological progress, PPMs remain invasive systems with limited longevity and a number of potential complications: infectious risks, mechanical failures, dyssynchronous pacing, and unresponsiveness to autonomic regulation [4].

The problem is particularly acute in paediatric practice and young patients, where regular device and lead replacements may become a source of severe complications [5]. These circumstances drive the search for alternative approaches capable of providing physiological myocardial stimulation without mechanical components.

One such direction is the development of biological pacemakers - cellular or gene constructs that can replace the function of the sinus node. An ideal biopacemaker should possess spontaneous electrical activity, ability to adapt to physiological load, safety, stable integration into the myocardium, and long-lasting effect [6].

The aim of this review is to analyse current approaches to the creation of biopacemakers, their functional characteristics, limitations, and clinical prospects.

Limitations of electronic pacing as a prerequisite for biopacemaker development

Despite continuous technological improvements, modern electronic cardiac pacing devices still have serious limitations and complications in clinical use. Among the problems associated with their use are:

1. Infection risk - about 12% of PPM implantations are complicated by device pocket infection or endocarditis. If system removal is required, risks increase sharply, especially in elderly patients with concomitant diabetes mellitus or immunosuppression. These situations require expensive treatment and carry a mortality rate of up to 10% [710];
2. Limited battery life - repeat surgeries carry additional risks;
3. Lead fractures or dislodgements - common causes of premature system failure. In young patients, this requires multiple reimplantations over a lifetime, associated with adhesion formation, venous thrombosis, and repeat surgical risks [11, 12];
4. Unresponsiveness to the autonomic nervous system - electronic devices do not fully adapt to autonomic regula-

tion; during physical or emotional stress, heart rate may not match metabolic demands, which is particularly critical in active patients;
5. Interference from other electrical devices (metal detectors, MRI magnets, power lines) that can affect PPM function;
6. Risk of heart failure due to chronic right ventricular pacing, especially with apical lead placement [13];
7. Paediatric problems related to the need to adapt the pacing system to the child's growth and development [14].

Even with considerable progress in device engineering, these limitations remain unresolved, highlighting the need for alternative approaches. Hence, the question arises about the creation of alternative pacing means, such as biological pacemakers.

Anatomy and physiology of the sinus node

The sinoatrial or sinus node (SAN) is the primary physiological pacemaker of the heart, initiating action potentials (AP) and ensuring the regularity of cardiac contractions. It is a heterogeneous specialised structure located subepicardially in the terminal groove, at the junction of the right atrium and the superior vena cava.

Cellular composition of the SAN:
• specialised pacemaker cells (Pcells) - the main rhythm generators, initiate automatic activity;
• transitional cells (Tcells) - ensure impulse conduction and transmission;
• fibroblasts - form and maintain the connective tissue framework (stroma of the SAN), surrounded by dense fibrous tissue.

Distinctive features of pacemaker and transitional cells are presented in Table 1.

The physiological basis of SAN function is automaticity - the ability to generate AP without external stimulation. The main electrophysiological feature of pacemaker cells is spontaneous diastolic depolarisation, driven by the interaction of several key ion currents and intracellular calcium mechanisms [15, 16].

1. Ion currents («membrane clock»):
• *If* ("funny current") through HCN1/4 channels is activated upon hyperpolarisation (from 100 to 40 mV) and provides the early phase of diastolic depolarisation due to Na^+ entry;
• *ICa,T* - Ttype calcium current (Cav3.1/3.2), activated at less negative potentials (50 mV) and contributing to further depolarisation;
• *INaCa* - sodiumcalcium exchanger current (NCX1), where extrusion of one Ca^{2+} is accompanied by entry of three Na^+;
• *ICa,L* - Ltype calcium current (Cav1.2/1.3), providing the rapid depolarisation phase of the AP;
• *IK* - potassium currents (particularly through ERG channels), responsible for repolarisation and restoration of resting potential [1719].

2 Calcium «clock»:
• spontaneous local Ca^{2+} releases (LCR) from the sarcoplasmic reticulum through RyR2;
• activation of NCX enhances membrane depolarisation and brings it closer to the AP threshold [20].

SAN automatic activity is strictly regulated by the autonomic nervous system. Sympathetic stimulation via βadrenergic receptors activates adenylyl cyclase, increases cAMP levels, which enhances HCN4 channel activity, increasing firing rate and heart rate. Conversely, parasympathetic stimulation (via acetylcholine and muscarinic receptors) suppresses these channels and slows heart rate.

Neurohumoral regulation:
• Sympathetic activation: β1receptors → ↑cAMP → activation of HCN4 and PKA → ↑If and ↑LCR → increased heart rate.
• Parasympathetic regulation: acetylcholine → M2receptors → ↓cAMP → ↓HCN4 and ↓Ca^{2+} entry → slowed rhythm.

Anatomically, the SAN artery plays an important role - most often it is the first branch of the right coronary artery. The node also has a sensory function due to its proximity to the aorta and dense autonomic innervation. The SAN artery not only nourishes pacemaker cells but also provides a direct link to systemic arterial pressure, allowing rapid responses to haemodynamic changes. Dense autonomic innervation, represented by both efferent and afferent fibres, forms reflex arcs for rapid rhythm regulation. Thus, the SAN functions not just as an isolated generator, but as an integrated sensory centre that perceives mechanical and chemical signals from atrial stretch and blood chemistry. This integrative function allows the SAN to finely adjust heart rate to the body's current metabolic demands [21, 22].

For normal SAN function, a delicate balance must be maintained between the relatively small number of pacemaker cells (source) and the surrounding atrial myocardium (sink), so that the SAN drives the larger surrounding myocardium without being overwhelmed by the hyperpolarising influence of the myocardium [23].

The presence of unique ion channels, such as HCN4, and weak electrical coupling with neighbouring cardiomyocytes allows the SAN to maintain its own potential and generate impulses without being depolarised by surrounding tissues. HCN4 channels act as an "electrical barrier": maintaining the Pcell membrane potential at 35...60 mV (vs. 90 mV in working cardiomyocytes).

Table 1.

Distinctive features of pacemaker and transitional cells

Characteristic	Central zone (P cells)	Peripheral zone (T cells)
Expression	HCN4↑, Cav1.3↑, NCX1↑	Cav1.2↑, Cx43↑
Function	Impulse generation	Impulse conduction
Unique features	Low Kir2.1, absence of Nav1.5	High electrical connectivity

Note: P cells - pacemaker cells; T cells - transitional cells; HCN4 - hyperpolarisation activated cyclic nucleotide gated channel type 4; Cav1.3 - voltage gated calcium channel L type, alpha 1D subunit; NCX1 - sodium calcium exchanger; Kir2.1 - inward rectifier potassium channel; Nav1.5 - integral membrane protein forming voltage gated sodium channel; Cav1.2 - voltage gated calcium channel L type, alpha 1C subunit; Cx43 - major gap junction protein in the heart, plays a critical role in synchronising cardiac contractions.

Low expression of connexin43 and its replacement by Cx30.2 and Cx45, which have higher electrical resistance, limit electrical coupling with atrial myocardium and contribute to functional isolation of the SAN.

The anatomy and ion profile of the SAN, including dominance of HCN4 (the subtype forming the pacemaker current If), weak coupling with the myocardium, and dual automaticity mechanisms, make it a reference model for the development of biological pacemakers. The dual oscillator system consists of membrane and calcium clocks, ensuring stable and rhythmic depolarisation processes. Diastolic depolarisation is formed by the pacemaker current If through HCN4 channels, early activation of Ttype Ca^{2+} current (ICaT), subsequent activation of Ltype Ca^{2+} current (ICaL), and reduction of potassium conductance (IK - repolarisation currents). In parallel, the calciumbased automaticity mechanism operates, based on cyclic Ca^{2+} accumulation by the sarcoplasmic reticulum, spontaneous local Ca^{2+} releases (LCR), and activation of the Na^{+}/Ca^{2+} exchanger (INCX), which adds a depolarising contribution to late diastole. Synchronous interaction of membrane and calcium "clocks" ensures a stable rhythm of spontaneous depolarisations. Weak electrical coupling of SAN cells with the surrounding atrial myocardium (low gap junction density, predominance of connexin45 - Cx45) reduces electrotonic load and enhances the efficacy of small depolarising currents, triggering the AP. Any cell or genebased strategy must consider these key parameters, including autonomic regulation and zonal organisation of the node.

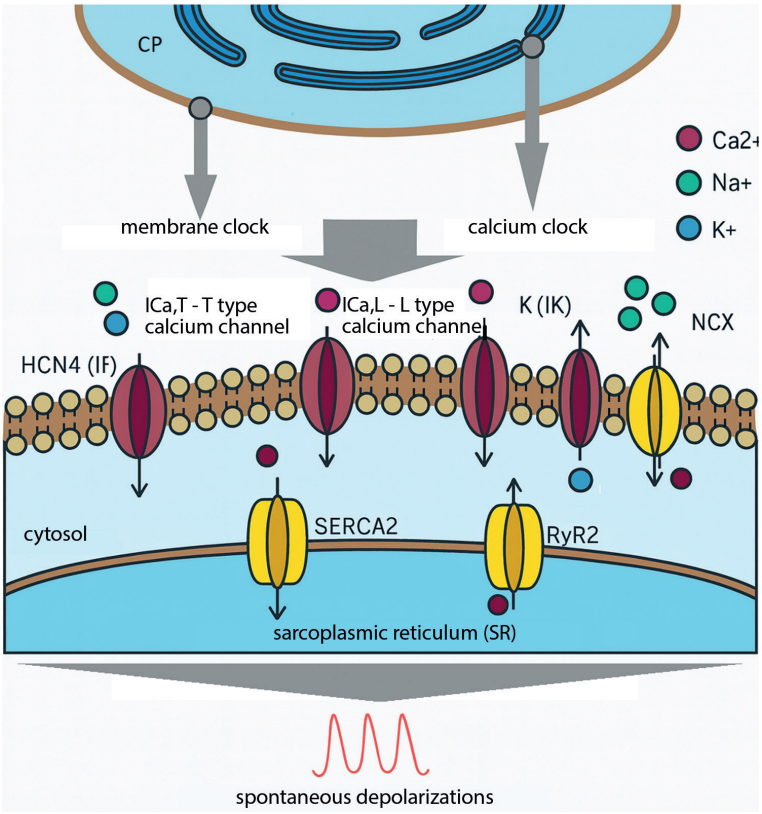

Fig. 1. Mechanism of sinus node automaticity. HCN4 - hyperpolarisation activated cyclic nucleotide gated channel type 4; If - funny current; NCX - sodium calcium exchanger; RyR2 - ryanodine receptor 2; SR - sarcoplasmic reticulum; SERCA2 - sarcoplasmic reticulum Ca^{2+} ATPase 2; ICaT - T type calcium current; ICaL - L type calcium current; IK - potassium currents.

Features of the SAN should be replicated in biological pacemakers. Ion channel proteins of membrane and calcium clocks must be expressed in biological pacemakers to provide sustained and reliable AP formation. Pacemaker cells should ideally be embedded in appropriate extracellular matrix proteins and surrounded by transitional cells and other key cell types. The sourcesink mismatch must be overcome, and APs should propagate to the surrounding myocardium; cells must be able to maintain rhythm for a long time. Finally, biological pacemakers must respond to autonomic influences (sympathetic and parasympathetic stimuli) to provide reliable rhythm generation in patients (Fig. 1).

HISTORY OF BIOPACEMAKER CONCEPT DEVELOPMENT

The history of biological pacemaker development dates back to the 1920s, with subsequent waves of research in the 1960s1980s. Early experiments attempted to use pieces of conducting tissue (e.g., the SAN itself), autografted, allografted, or xenografted into the ventricular myocardium. Autologous transplantation was most successful, sometimes restoring rhythm for 6 hours to 5 days. However, allo or xenotransplantation gave only temporary results due to inevitable fibrous degeneration.

The main problems that determined the failure of this approach were:

- pronounced immune reaction to foreign tissue;
- impaired blood supply leading to necrosis of the central part of the graft ("core necrosis");
- lack of functional electrophysiological integration with the recipient myocardium, preventing the graft AP from effectively exciting the cardiac muscle.

Thus, although these pioneering works showed the fundamental possibility of biological pacing, they clearly demonstrated the futility of simple mechanical transplantation of readymade bioexcitable conducting tissue. Awareness of these limitations became the next stimulus for the search for more refined and targeted rhythmrestoring technologies based on deep understanding of cell biology and electrophysiology [24-26].

MAIN STRATEGIES FOR CREATING BIOLOGICAL PACEMAKERS

The development of biological pacemakers represents an evolution from genetic modification of ion channels to the creation of autonomous cells capable of rhythmic depolarisation (Fig. 2).

Cellbased approaches

Use of native SAN cells: after failures with wholetissue transplantation, researchers moved to transplantation of isolated node cells, often mixed with atrial cardiomyocytes. The idea was that these cells, possessing innate electrogenicity, could become ectopic pacemakers upon establishing connection with recipient myocardium. A. Ruhparwar et al. demonstrated electrical coupling of foetal

cardiomyocytes in dogs; G. Lin et al. achieved functional connection of human cells in porcine hearts; H. Zhang et al. revealed a strong influence of the microenvironment on phenotype and activity of transplanted autologous SAN cells in dogs [2729]. These works proved the principal feasibility of biopacing but did not lead to clinical success, stimulating the development of stem cellbased approaches.

Modern approaches to creating biological pacemakers are based on regenerative medicine methods, particularly using embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). ESCs and iPSCs are considered the most promising cell source due to their ability to differentiate into any cardiomyocyte type, including pacemaker cells. Differentiation protocols mimic embryonic heart development in vitro (formation of embryoid bodies, 2D cultures with biochemical signals). The key problem remains obtaining pure populations of SANlike cells.

The work of I. Kehat et al. first demonstrated the possibility of differentiating ESCs into functional cardiomyocytes that not only engrafted in pig hearts but also functionally stimulated contractions [30]. An important step was made by T. Xue et al., who recorded and confirmed direct spread of depolarisation wave from the graft to the myocardium [31]. The group of S.I. Protze et al. achieved generation of SANlike cells from human iPSCs without genetic modification, using only combinations of growth factors (BMP4, Activin A, etc.) [32].

To enhance specificity, chemical treatments (e.g., 1EBIO), fluorescently labelled lines (e.g., selection of *NKX2.5/ISL1*+ progenitors), transgenefree protocols, and CRISPR platforms (e.g., selection of *TBX5+/NKX2.5* cells) are used. Clinical steps were taken in the ESCORT study with ESCderived progenitors in heart failure; and in the Japanese study NCT04696328 with allogeneic iPSCs in ischaemic cardiomyopathy [33, 34].

Genebased approaches

The SAN develops from a group of progenitor cells that express the *Tbx18* gene. This gene acts as a master regulator, directing these cells into right atrial structures, including the SAN itself. The further fate of pacemaker cells depends on another key factor - the *Shox2* gene. It initiates a cascade of genes (including *Isl1* and *Tbx3*) that regulate the synthesis of HCN4 channel proteins, Tchannels, gap junction proteins connexin Cx30.2, etc. It is this set of proteins that allows the cell to generate the cardiac impulse and transmit it to neighbours [35] (Fig. 3).

Genebased approaches are based on the idea of "teaching" ordinary cardiac muscle cells to perform the pacemaker function. For this purpose, viral or nonviral vectors are used to deliver genes responsible for generating electrical impulses to the myocardium. The main strategies can be outlined (Fig. 4).

Suppression of IK1 current - "unlocking" latent automaticity in ordinary cardiomyocytes. Miake et al. first showed that suppression of *Kir2.1* gene expression (via dominantnegative Kir2.1AAA) in cardiomyocytes leads to diastolic depolarisation similar to SAN cells, "unlocking" latent automaticity [36].

Expression of β2adrenergic receptor genes - increasing sensitivity to natural rhythm stimuli. Edelberg et al. showed that transfer of the human β2adrenoceptor gene into the atrium increases heart rate by 4050% due to enhanced catecholamine sensitivity [37, 38]. However, this approach provides only a chronotropic effect, not full rhythmogenesis.

Expression of *HCN* family genes (hyperpolarisationactivated cyclic nucleotidegated). The *HCN* genes, encoding channels of the "funny" current (If) - hyperpolarisationactivated cation channels - play a central

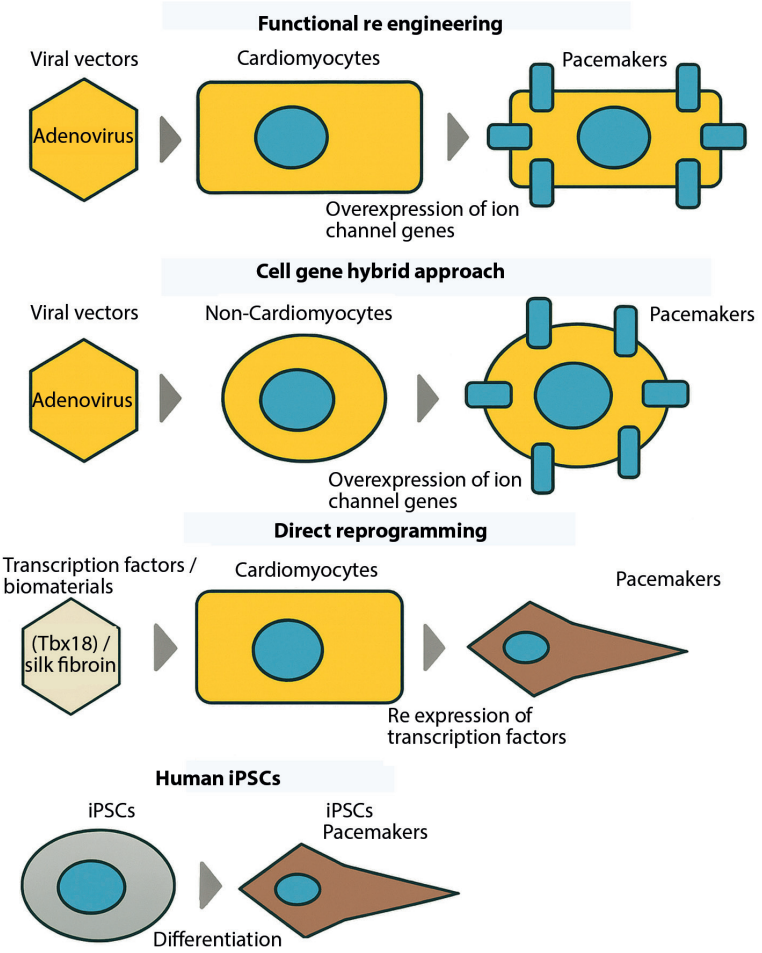

Fig. 2. Approaches to creating biological pacemakers. Tbx18 - T box transcription factor 18, plays an important role in pacemaker cell differentiation; iPSC - induced pluripotent stem cells; functional re engineering - use of adenoviral vectors for overexpression of genes encoding ion channels in cardiomyocytes to create automaticity; cell gene hybrid approach - use of adenoviral vectors for overexpression of genes encoding ion channels in non cardiomyocytes (mesenchymal, iPSCs, fibroblasts) to create automaticity; direct reprogramming - changing cardiomyocytes into pacemaker cells with integral changes in morphology, structure, function, and transcription through re expression of transcription factors (Tbx18) or biomaterials (e.g., silk fibroin); human iPSCs - differentiation of human iPSCs or embryonic stem cells into pacemaker cells.

role in forming spontaneous diastolic depolarisation in SAN cells.

Experimental data from largeanimal models confirm the functional efficacy of *HCN* gene modifications:

- delivery of the *HCN2* gene restored heart rate during SAN arrest, demonstrating the ability to restore stable cardiac rhythm in a canine model of vagal SAN arrest [39];
- use of a modified *HCN1* gene created a stable rhythm in pigs with sick sinus syndrome [40].

To make the new rhythm not only stable but also physiological (i.e., accelerating under load), genes are combined:

- *HCN2* combined with the sodium channel gene (*SkM1*) improves impulse conduction [41];
- combination of *HCN2* with the adenylyl cyclase gene allows creation of a rhythm that responds to autonomic signals, accelerating under stress like the natural one [42].

MicroRNAbased therapy. This is a newer, more refined approach. MicroRNAs are molecules that regulate gene function. It has been found that in some forms of SAN dysfunction, the activity of microRNAs (*miR4235p* and *miR3703p*) that suppress the main pacemaker gene *HCN4* is increased. The strategy of inhibiting pathologically elevated expression of *miR4235p* and *miR3703p* with antimiR oligonucleotides opens new possibilities for pathogenetic therapy of SAN dysfunction by restoring physiological *HCN4* expression and normalising pacemaker activity [43].

While direct *HCN2* gene delivery induces If current formation in the myocardium, a more fundamental approach is to reprogramme the phenotype of mature cardiomyocytes. The most promising and fundamental approach is not just to add one gene, but to “retrain” the mature cell, making it recall its embryonic past. For this, the *TBX18* gene is used - the main conductor of SAN development during the embryonic period. The study by N. Kapoor et al. became one of the proofs of this concept,

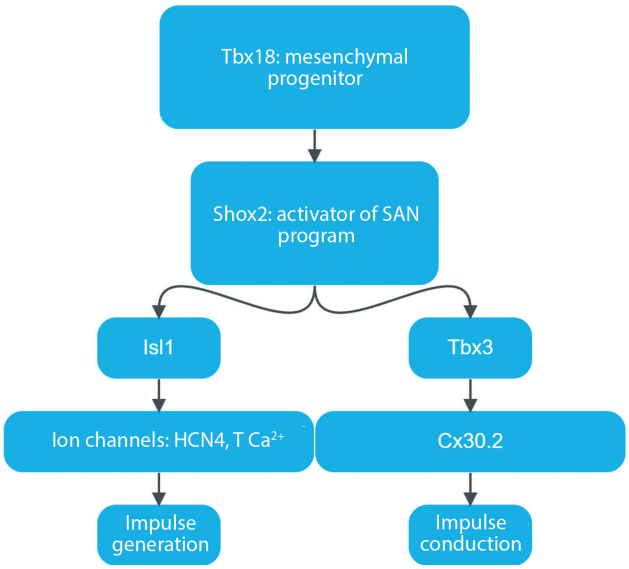

Fig. 3. Embryonic formation of the sinus node. Tbx18 - marker of sinoatrial node progenitor cells; Shox2 - factor necessary for sinoatrial node development; Isl1 - participates in formation of HCN4, T Ca^{2+} ion channels of the heart, ensuring impulse generation; Tbx3 - activation of Cx30.2 ensures impulse conduction.

demonstrating that delivery of *TBX18* into ordinary cardiomyocytes turns them into pacemakerlike cells with all necessary properties [44].

The study by Y.F. Hu et al. showed that delivery of the *TBX18* gene into the ventricular myocardium of pigs with complete AV block led to generation of a stable idioventricular rhythm that persisted throughout the observation period (14 days). These results support the potential of *TBX18*mediated reprogramming as a strategy for creating biological pacemakers [45].

Combined genecell approaches

The strategy of cellgene therapy is a combined approach involving transplantation of carrier cells (most often mesenchymal stem cells (MSCs) or cardiac progenitor cells (CPCs)) genetically modified to express key pacemaker ion channels, such as HCN1, HCN2, HCN4, and the skeletal muscle sodium channel SkM1. In this context, cells function as biological platforms for targeted and temporally controlled delivery of ion channels to the target myocardial zone.

In vitro and in vivo experiments have confirmed the feasibility of this approach. Transplantation of human MSCs expressing HCN2 induced pacemaker activity in cell culture and restored rhythm in dogs with a bradyarrhythmia model, with the effect lasting up to 6 weeks [46, 47]. Similarly, MSCs modified with *HCN1* and *HCN4* genes demonstrated the ability to influence contraction synchronisation of rabbit cardiomyocytes in vitro and induce stable idioventricular rhythm in vivo in rats and rabbits [4850]. An alternative strategy using SkM1 normalised conduction velocity in the postinfarction scar zone in dogs without provoking arrhythmias [51]. To improve integration and longterm survival of transplanted cells, human CPCs coexpressing *HCN2* and *SkM1* were used, showing higher efficacy than MSCs [52]. Furthermore, the principal possibility of creating functional hybrid cells with pacemaker properties by fusing *HCN1*expressing fibroblasts with cardiomyocytes was demonstrated [53].

Despite promising results, the method faces several technological challenges, including the risk of migration of transplanted cells from the implantation site, for which cell encapsulation in biocompatible hydrogels is being explored. The problem of insufficient electrical coupling between carrier cells and cardiomyocytes is addressed by using electromechanically integrating CPCs. The potential oncological risk associated with viral vectors stimulates the development of nonviral delivery systems and optimisation of constructs for transient and controllable transgene expression.

Reprogramming of somatic cells with transcription factors

The most fundamental approach to creating biological pacemakers is the direct reprogramming of mature somatic cells, such as cardiomyocytes or fibroblasts, into cells with a pacemaker phenotype. This strategy is based on inducing the expression of key transcription factors that regulate embryonic SAN development, allowing direct reprogramming of the target cell transcriptome.

The most effective factor in inducing the pacemaker phenotype has been *TBX18*. The study by N. Kapoor et al. showed that *TBX18* expression in rat ventricular car-

diomyocytes in vitro initiates their transdifferentiation into cells morphologically and functionally similar to SAN cells [44]. This process was characterised by suppression of genes typical of working myocardium (including the sodium channel Nav1.5 and connexin43 (Cx43)) and simultaneous activation of *HCN4* expression, generation of pacemaker If current, and appearance of spontaneous pacemaker automaticity. Importantly, in vivo delivery of *TBX18* to the ventricular myocardium of pigs with complete AV block resulted in a stable idioventricular rhythm.

In addition to *TBX18*, several other critical regulators exist:

- *SHOX2* acts upstream in the reprogramming cascade, suppressing expression of the transcription factor Nkx2.5 - a repressor of SAN pathway differentiation - and activating *HCN4*and *TBX3* expression;
- *TBX3* plays a key role in maintaining the pacemaker phenotype by actively repressing the working myocardial gene program;
- *ISL1* serves as a marker of conduction system progenitor cells and is important for their development and potential cell regeneration;
- *TBX5* is a key regulator of atrioventricular node development.

To enhance efficiency and completeness of reprogramming, strategies of coexpressing several factors, e.g., combinations of *SHOX2*, *HCN2*, and *TBX5*, are being investigated [53]. The main advantage of this approach is the possibility of local creation of a biological pacemaker without cell transplantation. Relative safety and reversibility can be ensured by using nonintegrating vectors for gene delivery. However, the approach also faces serious challenges, such as risk of incomplete or heterogeneous reprogramming, potential oncogenicity associated with viral vectors, and the need to develop systems for precise spatiotemporal control of transcription factor delivery and expression.

Problems and limitations identified at the development stage

Despite impressive preclinical successes, no biological pacemaker has yet been introduced into clinical practice. The main barriers include:

Safety (risk of ectopic activity and lifethreatening arrhythmias from offtarget delivery or uncontrolled expression, potential immunogenicity of transplanted cells or vectors, oncological risks associated with viral vectors (insertional mutagenesis) and longterm culture / use of PSCs (genomic instability).

Stability and durability of effect. A biopacemaker must provide reliable, physiologically synchronised activity for years. Most preclinical effects (especially gene therapy) last weeks to months, incomparable with decades of electronic device operation. Causes include immune elimination, loss of gene expression, cell dedifferentiation.

Control. Data obtained in rodents, whose electrophysiology (high heart rate, ion currents) differs substantially from human. Longterm studies in large animals (pigs, sheep) are needed, considering differences in microenvironment, metabolism, and innervation.

Reversibility. Lack of reliable mechanisms for control, modulation, or complete shutdown of biopacemaker activity, particularly critical for gene therapy.

Ethical issues. Particularly relevant for ESCbased approaches.

OWN EXPERIENCE IN BIOPACEMAKER DEVELOPMENT (20162018)

Within a project supported by the Russian Science Foundation (No. 161510322), our centre's team obtained

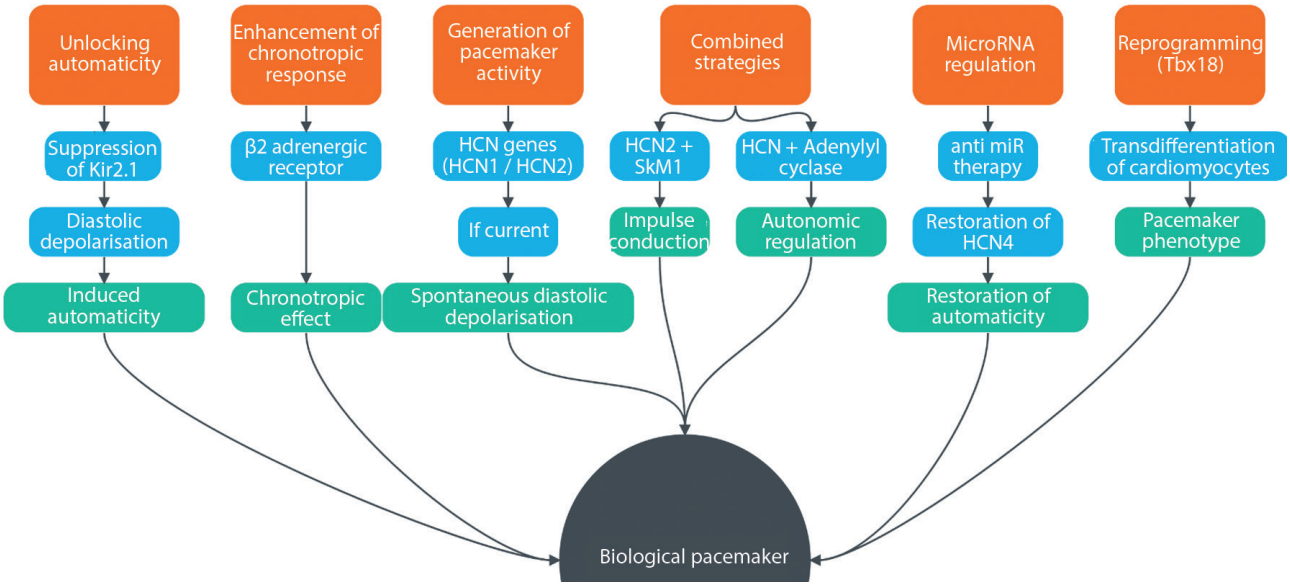

Fig. 4. Genetic strategies for creating a biological pacemaker. Kir2.1 (IK1): potassium channel protein forming the IK1 current (if blocked, working cardiomyocytes may begin to spontaneously generate impulses, like pacemaker cells); HCN genes (HCN1, HCN2, HCN4) - gene family encoding channels that conduct the If current; If current - the main pacemaker current flowing through HCN channels; HCN2 provides the slow diastolic depolarisation phase (If current), while SkM1 (skeletal sodium channel) adds a fast inward sodium current; adenylyl cyclase - enzyme that produces cyclic AMP, which directly binds to HCN channels and shifts their activation curve positively, causing channels to open more frequently; anti miR therapy - a modern therapeutic method using synthetic oligonucleotides to inhibit pathological microRNAs.

a set of significant results that form the basis for developing a new generation of tissueengineered biological pacemakers.

Key achievements

Engineering of biomimetic polymer matrices. Optimisation of electrospinning technology allowed the creation of nanofibrous scaffolds with controlled architecture based on polycaprolactone, polylactic acid (PLA), and recombinant spider silk (spidroin). Surface modification with fibronectin was found to statistically significantly enhance cardiomyocyte adhesion, while spidroinbased matrices demonstrated superior elastic properties without additional processing. Functional competence of the constructs was confirmed in experiments with neonatal rat cardiomyocytes, which, when cultured on these matrices, maintained synchronous contractile activity and conduction, visualised by optical mapping using the calcium indicator Fluo4.

Differentiation of pacemaker cells from iPSCs. Protocols for directed differentiation of human and rat iPSCs into pacemakertype cardiomyocytes were developed via modulation of the WNT/βcatenin signalling pathway (using CHIR99021 and IWP2 porphyrin). The efficiency of differentiation into pacemaker cells (*TnT+/Nkx2.5* phenotype) was increased from 0.5% to 14% through synergistic action of the morphogen BMP4 and sitespecific activation of key transcription factors SHOX2 and TBX5 using the CRISPR/Cas9 system. To create a controllable pacemaker system, hybrid cell cultures were formed combining the obtained iPSCderived cardiomyocytes with optically controllable ChR2HL1 cardiomyocytes, which demonstrated stable rhythmic activity in response to light stimulation.

Development of transplantation and integration technologies. To improve survival of transplanted cells, biocompatible micropatterns based on PLA/collagen composite were developed. It was experimentally shown that using thrombin gel as a carrier for cell suspension extended the persistence of cardiomyocytes in porcine myocardium to 10 days, twice as long as transplantation without carrier (5 days). In preclinical studies in minipigs, transplanted iPSCderived cardiomyocytes did not induce ectopic activity, but enhanced immunosuppression was required to prevent rejection.

Future directions

The accumulated data allowed the formation of a prototype platform for a tissueengineered biological pacemaker, integrating three key components: (1) biocompatible matrices with controlled architecture, (2) optimised cell products, including lines with optogenetic control, and (3) targeted delivery methods ensuring improved integration with recipient tissue. The strategic direction of further research is to enhance pacemaker phenotype stability and ensure longterm functional activity of the constructs in vivo [54-57].

Thus, the studies conducted in 20162018 created a scientific and technological foundation for further development of biological pacemaker research. The obtained results validated key tissue engineering and cell biology approaches for cardiac pacing, and also identified priority directions for subsequent work aimed at improving pacemaker phenotype stability, enhancing myocardial integration, and transitioning from shortterm preclinical models to longterm in vivo studies.

CONCLUSION

The development of biological pacemakers remains one of the most dynamic and promising areas of regenerative cardiology, integrating embryology, molecular biology, genetic engineering, and tissue engineering. Significant evolution has occurred: from primitive SAN tissue transplantation, through genetic engineering and iPSC technologies, to complex combined delivery and direct cell reprogramming methods.

Although clinical translation requires overcoming serious safety and efficacy barriers, the experimental evidence is compelling. The future likely lies not in complete replacement of electronic pacemakers, but in their integration with biological components or the use of biopacemakers in specific niches:

1. In patients with contraindications to implantation (infections, vascular anomalies);
2. As a temporary solution (e.g., in children requiring pacing until adulthood);
3. In combination with microelectronics and biosensors to create “smart” hybrid systems maximally adaptive to the body’s needs;
4. Use of allogeneic “universal” iPSC lines with low immunogenicity.

Modern technologies (CRISPR editing, cardiac organoids, 3D bioprinting, biodegradable scaffolds, targeted delivery) are actively being explored to solve existing problems. Despite difficulties, clinical application of biopacemakers appears achievable in the foreseeable future.

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